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Screening the Biometabolites of *Pterocarpus santalinus* L.F.-An Endemic, Threatened, Medicinal and Multipurpose Plant Taxon.

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Abstract
Pterocarpus santalinus L.f. commonly known as red sanders belongs to the family Fabaceae. It is an endemic to Sheshachalam hills at Nallamala forest of Eastern Ghats, India and considered globally endangered. Due to its high medicinal and economic value and illegal harvest of wood and exports to other countries is being a key threat. Phytochemical constituents are responsible for medicinal value of plant species. Hence in the present study Qualitative and Quantitative screening of biometabolites were carried out separately from leaves, stem bark, shoot tips and auxiliary buds of *P.santalinus*. The presence of various biometabolites like Flavonoids, Tannins, Saponins, Alkaloids, Phenols, Terpenoids, Steroids, Glycosides, Carbohydrates and Proteins were found in polar and non-polar solvents. Aqueous extract consists of a greater number of biometabolites than that of other extracts of stem bark, axillary buds, shoot tips followed by leaves. High quality of phenols and tannins were found in leaf and stem bark respectively. The Results Suggested that the presence of various bioactive compounds in different concentrations confirm the applications of *P.santalinus* against many ailments. All parts of *P.santalinus* are highly potential for application in traditional medicines as well as for isolation of novel drugs through Pharmaceutical industry.

Keywords
Pterocarpus santalinus, Endangered, phytochemicals, novel drug, and pharmaceutical industries.

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INTRODUCTION
Medicinal plants are the richest bio-resources of folk medicine; traditional systems of medicine; food supplements, nutraceuticals, pharmaceutical industries and chemical entities for synthetic drugs [1]. Modern medicine has evolved from folk medicine and traditional systems only after through chemical and pharmaceutical screening [2]. India is the birthplace of renewed systems of indigenous medicine such as Siddha, Ayurveda, and Unani. Traditional systems of medicines are prepared from a single plant or combinations of many plants. The natural potency of phytocompounds depends on the choose and use of proper plant part and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolite in the raw drug [3]. There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity [4]. Screening of active compounds from plants has led to the invention of new medicinal drugs which have efficient protection and treatment against various diseases, including cancer [5] and Alzheimer [6]. Phytochemicals are basically divided into two groups that is primary and secondary metabolites based on the function in plant

metabolism. Primary metabolites are comprised common carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and so on [7-8]. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs. There is an increasing interest in the phytochemical compounds, which could be relevant to their nutritional incidence and their role in health and disease. Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available and less side effects [9]. Number of plants were studied for qualitative and quantitative phytochemical analysis for their medicinal values of number of plants like *Svensonia hyderobadensis* [10], *Boswellia ovalifoliolata*[11], *Shorea tumbuggaia* [12]. *Allamanda catharitica* [13] and *Cochlospermum religiosum* [14]. *Syzygium alternifolim* [15] *Terminalia pallida* [16], *Dysophylla myosuroides* and *Talinum cuneifolium* [17], *Clinacanthus siamensis* and *Cissampelos pareira* [18], *Abrus precatorius, Adhatoda vasica* and *Catharanthus roseus* [19], *Adansonia digitata* [20], *Ficus mollis* [21] and *Nymphaea rubra* [22] and Underutilized Species of Cyperaceae [23].

The selected medicinal plant *P.santalinus* L.f. (Fabaceae) **(Fig 1)** is commonly known as Red sanders and an endemic medicinal tree in India and considered globally endangered [24]. A red sander has fallen back into the endangered category in the IUCN Red list -2022.it was classified as near threatened in 2018. The Union Ministry of Environment, Forest and climate change (MoEFCC) 2022 jan8 th assessed as endangered. The slow growth of species and continued harvesting of trees has no time for the species to recover naturally.

Fig.1 *Pterocarpus santalinus*

The colour and fragrance of *P. santalinus* heartwood are derived from santalins while the pleasent aroma is caused by the presence of terpenoids [25]. A dye prepared from the heartwood *of P. santalinus* is used as a stain in light microscopy [26], as a coloring agent in pharmaceutical preparations, in food, leather and textile industries [27], and as a textile dye [28]. The texture and colour differentiate good quality from poor quality trees, with ‘‘wavy grain wood texture with intense red color’’ in the former and ‘‘straight grain wood texture with light red color’’ in the latter and it is the superior quality of *P. santalinus* that makes it popular in the furniture industry [29]. In Japan, *P. santalinus* is used to make carvings and musical instruments, shamisen and koto [30], as well as name seals or hankos. In Buddhism, *P. santalinus* is considered to be a symbol of holiness, and is thus used for carved statues, as a constituent of incense [31], and for cremation [32]. In China, *P. Santalinus*

wood has a long history of use in furniture and other valuable wood products [33].

In the traditional system of medicine, the decoction prepared from the heartwood is attributed various medicinal properties. It has been used in inducing vomiting and treating eye diseases, mental aberrations and ulcers. The heartwood of red sanders is known to have antipyretic, anti-inflammatory, anthelmintic, tonic, hemorrhage, dysentery, aphrodisiac, diaphoretic activities, and cooling agent. Ethanol extract of stem bark was reported to possess anti-hyperglycaemic activity. The wood in combination with other drugs is also prescribed for snake bites and scorpion stings [24]. *P. santalinus* is a highly impressive indigenous species [34], thus researchers during the past two decades have shown a renewed interest [35-36]. The heartwood is rubbed with water, honey, ghee, and oil, applied as collyrium to alleviate defects of vision. It is also used for treating skin diseases, bone fracture, leprosy, spider poisoning, hiccough, ulcers, general debility, and mental aberrations [37]. A natural dye, santalin, extracted from red sanders wood is used for colouring pharmaceutical preparations, food stuffs etc. Extracts of wood and fruit find extensive applications as astringents, diaphoretics, external applications for inflammations, headache, skin diseases, bilious infections, and chronic dysentery [38]. The medicinal values of a species are attributed to its biometabolites. Moreover, each part of the same plants shows variations in its active principles. Qualitative and quantitative analysis of biometabolites of endemic and endangered medicinal plants are scanty. Hence the present study is selected to screen the plant parts for their qualitative and quantitative bioactive compounds separately to identify the efficient and potential plant parts for further studies like isolation of novel drugs.

MATERIAL AND METHODS

P. santalinus is found exclusively in a well-defined forest tract of Andhra Pradesh in Southern India [39], but is also found in the Chinese provinces of Yunnan, Guangdong and Guangxi, and on Hainan Island, where it is referred to as zitan [40].

P.santalinus is a large deciduous tree; bark exudes blood red juice on incision, Leaves 3 foliate, leaflet very rarly 2 pairs, coriaceous, entire, obtuse. Flower yellow in auxiliary racemes panicles. Standard ovate petal, stamens 10, pods obliquely orbicular, becoming narrowed into short stalk, narrowly winged [41].

Plant material collection

The fresh plant materials were collected from the sri venkateswara university campus of Tirupati, Andhra Pradesh, India.

Preparation of plant extract for Qualitative analysis:

Leaves, stem bark, axillary buds and shoot tips of *P. santalinus* were collected and washed with water, chopped into small fragments, shade dried and made into coarse powder which was passed through a 40-mesh sieve to get uniform particle size and stored in amber colour bottles separately each sample of 100 gm of powder was subjected to continuous hot extraction in soxhlet apparatus individually with aqueous, acetone, chloroform, diethyl ether and methanol. The residue was separated; the extract was filtered through Whatmann filter paper No-1, and evaporated under reduced pressure using a rotary evaporator until all the solvent had been removed to obtain the extracts. The obtained extracts were preserved in refrigerator at 4^{o}C to avoid any contamination until further study.

Preliminary Phytochemical Screening

The above obtained soxhlet extracts were used for Preliminary phytochemical testing nearly 13 components namely flavonoids, steroids, tannins, glycosides, saponins, alkaloids, phenols, anthraquinones, anthocyanins, coumarins, lignins, proteins and Triterpenoids was done by the standard procedures described by Harborne, 1984 [42] and Kokate *et al.,* 1991[43].

Quantitative Phytochemical Analysis:

Quantitative analysis of biometabolites was Carried out by using standard procedures for proteins; Lowry *et al.,* 1951 [44], carbohydrates; Krishnaveni *et al.,*[45], phenols; Sadasivam and Manickam 1992 [46], flavonoids; Zhishen *et al.,*1999 [47], saponins; Peng and Kobayasli 1995[48], alkaloids; Higuchi and Bodin 1961 [49] and tannins; Price and Butter 1997 [50].

RESULTS AND DISCUSSION

Qualitative and quantitative analysis (Table-1)

Results revealed that stem bark yield a good number of compounds in Methanol. The methanolic extract of stem bark showed the presence of flavonoids, steroids, amino acids, tannins, glycosides, saponins, phenols, alkaloids, anthroquinones, Anthocyanins Lignin and triterpenoids. Aqueous extract showed the presence of flavonoids, glycosides, saponins, alkaloids, phenols, Coumarins and lignins followed by Acetone extract showed the presence of steroids, glycosides, saponins, alkaloids and phenols. Whereas in the chloroform and Diethyl ether extracts only flavonoids, Glycosides, saponins, phenols and steroids were present. Among all the solvents,

methanol is the best suitable solvent for extracting *P.santalinus* bioactive compounds. Similar type of results were observed in *Svensonia hyderobadensis*[10], *Boswellia ovalifoliolata*[11], *Shorea tumbuggaia* [12], *Allamanda catharitica* [13], *Cochlospermum religiosum* [14]. *Syzygium alternifolim* [15] *Terminalia pallida* [16], *Dysophylla myosuroides* and *Talinum cuneifolium* [17], *Clinacanthus siamensis* and *Cissampelos pareira* [18], *Abrus precatorius, Adhatoda vasica* and *Catharanthus roseus* [19], *Adansonia digitata* [20]. All previous studies carried out by many researchers on *Pterocarpus* revealed that methanol and aqueous extracts are the best solvents for phytochemical analysis. Our research work on phytochemical screening also supports the same results.

The quantitative estimation of secondary metabolites of *P.santalinus* stem bark, Leaves, Shoot tip and Axillary buds showed rich in alkaloids, flavonoids, phenols, sapoinins tannins, proteins and carbohydrates. The results are summarized in **Table-2** and highest number of alkaloids 0.617 mg/g d.wt. are present in shoot tip. Alkaloids are beneficial chemicals to plants with predators and parasite repelling and physical state. Number alkaloids are isolated from dicots and using efficient drugs. The alkaloids are one of the most diverse groups of secondary metabolites found in living organisms and have an array of structure types, biosynthetic pathways and pharmacological activities. The presences of alkaloids contained in plants are used in medicine as aesthetic agents [51]. Stem bark is rich in phenols 1.893 mg/g dw. and Flavonoid 1.713 mg/g dw. The higher amounts of phenols are important in the regulation of plant growth, development, and disease resistance. Over the last few years, several experimental studies have revealed biological and pharmacological properties of phenolic compounds, especially their anti-inflammatory activity, antiviral and cytotoxic activity [52]. Flavonoids are reported to possess many useful properties, including anti-inflammatory, antimicrobial, enzyme inhibition, oestrogenic, antiallergic, antioxidant and anti-tumour activity [53, 54]. Highest amount of Tannins are present in Leaves 1.997 mg/g dw. Leave confined maximum amount of tannins. Tannins contribute the property of astringent activity i.e., faster the healing of wounds and inflamed mucous membrane [55, 56]. Saponins 0.502 mg/g dw, Proteins 0.413 mg/g dw and Carbohydrates 0.211mg/g dw. are in Stem bark as well as leaves which are primary components of living organisms. Proteins are essential to maintain the structure and functions of all life and vital for growth and development. The presence of higher protein level in the plants points towards their possible increase in food value or that a protein based bioactive compound could also be isolated in future. Carbohydrates are one such group of carbon compounds, which are essential to life. [57]. Traditionally saponins have been extensively used as detergents as pesticides and mollusicides, in addition to their industrial applications as foaming and surface-active agents and also have beneficial health effects [58].

Table 1: Qualitative Analysis of biometabolites from various parts of *P. santalinus.*

S.No	Tests	Aqueous				Acetone				Chloroform				Diethyl ether				Methanol			
		L	SB	AB	ST	L	SB	AB	ST	L	SB	AB	ST	L	SB	AB	ST	L	SB	AB	SP
1	Flavonoids	+	+	+	+	+	+	-	+	+	-	+	+	+	-	+	-	+	+	+	+
2	Steroids	+	+	-	+	+	+	+	-	+	-	-	+	+	-	+	-	+	+	+	+
3	Tannins	+	+	-	+	+	+	-	-	-	+	-	-	-	-	-	-	+	+	+	+
4	Saponins	+	-	+	-	+	-	+	-	-	+	-	-	-	+	+	+	+	+	+	+
5	Alkaloids	-	+	+	-	+	+	+	-	-	-	-	-	-	+	-	-	+	+	+	+
6	Phenols	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+	+
7	Triterpenoids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
8	Anthraquinones	+	+	-	-	-	+	-	-	+	-	-	-	+	-	+	-	+	+	+	+
9	Anthocyanins	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
10	Coumarins	+	-	+	+	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-
11	Lignins	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-
12	Proteins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+
13	Glycosides	-	+	+	+	-	+	+	+	-	+	+	+	-	+	-	-	+	+	+	+
14	Total	07	09	07	07	06	07	05	03	03	03	02	04	03	04	05	03	10	12	11	10

L- Leaf, SB-Stem Bark, AB-Axillary Buds and ST-Shoot Tips.
+ indicate presence & - indicates absence.

Table 2: **Quantitative analysis of biometabolites from different parts of *P.santalinus* mg/g D.W.**

S.NO	Phytochemicals	Stem bark	Leaf	Shoot tip	Axillary bud
1	Alkaloids	0.225±0.012	0.412±0.206	0.617±0.088	0.114±0.088
2	Flavonoids	1.713±0.088	1.331±0.88	0.322±0.243	0.333±0.088
3	Phenols	1.893±0.028	1.641±0.088	1.839±0.057	0.371±0.057
4	saponins	0.502±0.088	0.502±0.088	0.413±0.088	0.362±0.088
5	Tannins	1.231±0.37	1.997±0.088	0.415±0.027	0.052±0.057
6	proteins	0.413±0.057	0.413±0.057	0.042±0.057	0.212±0.021
7	Carbohydrates	0.211±0.057	0.211±0.088	0.031±0.088	0.075±0.057

Values are average of triplicates ±, SE

CONCLUSION

Standardization of herbal drugs should be ensured to provide sound scientific footing to enhance consumer confidence and to improve business prospects for herbal medicines. The medicinal plants appear to be rich in secondary metabolite, widely used in the traditional medicine to combat and cure ailments. The anti-inflammatory, antipyretic, anthelmintic, tonic, hemorrhage, dysentery, aphrodisiac, and diaphoretic activities can be attributed to their high phenols, flavonoids, tannins and alkaloids. Exploitation of these pharmacological properties involve further investigation of these active ingredients by implementation of extraction techniques like extraction, purification, separation, crystallization and identification. The present work was thus planned to establish pharmacognostic standards for *P.santalinus* so as to have reliable parameters to authenticate the plant for its medicinal values.

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